Lymphocyte homing: **The scent of a follicle**

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The recent discovery of a receptor needed for lymphocyte migration into lymphoid follicles indicates that multiple chemoattractive gradients allow lymphocytes to navigate to specialized niches in lymphoid organs.

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Following an infection, the swift eradication of microorganisms from the body depends on the orchestrated migration and congregation of diverse cells of the immune system. The focal points for immunological congregation are the spleen, lymph nodes, Peyer's patches and other so-called secondary lymphoid tissues. Within these organs, specialized cell migration routes and microenvironmental niches exist for presenting infectious antigens to a torrent of recirculating lymphocytes, thus enabling rare T and B lymphocytes carrying specific antigen receptors to be quickly selected and clonally expanded as immune effector cells. Very little is known about the molecular signposts that guide the traffic to discrete compartments of secondary lymphoid tissue at different stages of the immune response, despite clear evidence that understanding those cues holds important keys to acquired immunodeficiency syndrome (AIDS), transplantation, autoimmune disease and metastatic cancers. A recent paper by Förster *et al.* [1] represents a turning point in the field, illuminating an important role for an orphan chemokine receptor, Burkitt's lymphoma receptor 1 (BLR1), in guiding lymphocytes to lymphoid follicles in spleen and Peyer's patches.

The anatomy of immune responses

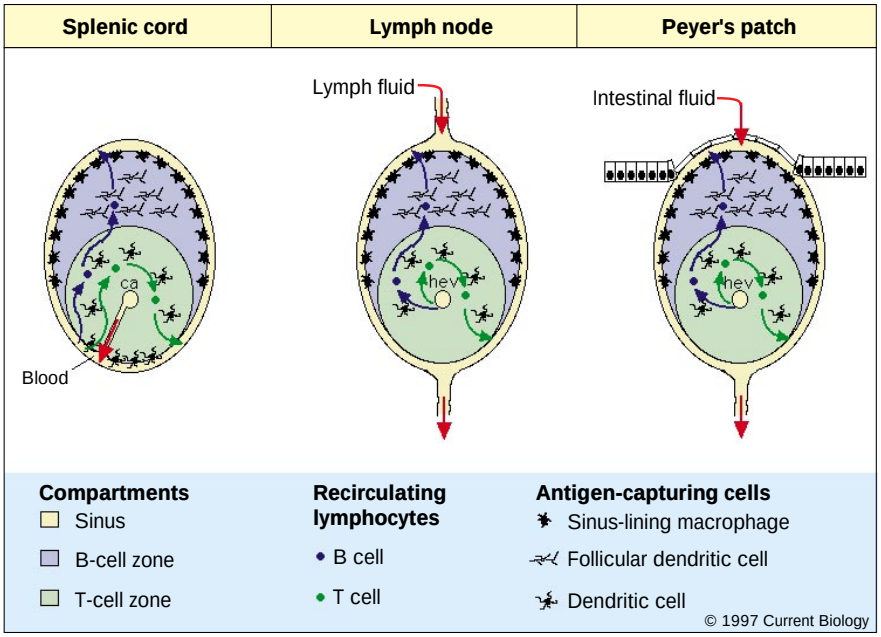
Like all areas of anatomy, coming to grips with the byzantine architecture and circulatory patterns of secondary lymphoid tissues depends on matching arcane names with important functions. The central function of secondary lymphoid tissues is filtering: collecting blood-borne, lymph-borne or mucous-membrane antigens and holding them so that they can be surveyed by immune cells before being destroyed. In the spleen, blood is filtered as it percolates slowly through branching marginal zone sinuses (Fig. 1). Lymph fluid draining from normal or inflamed tissues is filtered as it slakes through the subcapsular and cortical sinuses of lymph nodes. Intestinal antigens are sampled in the Peyer's patches after transport across specialized epithelial cells called M cells.

The antigen-collecting function of secondary lymph tissues is aided by specialized antigen-capturing cells, such as Langerhans/dendritic cells and marginal zone/sinus-lining macrophages, which are poised to bind and/or internalize microorganisms and their antigens. These cells wait strategically at potential sites of infection: within tissues, beneath the epithelium of Peyer's patches, or along the linings of the sinuses in the spleen and lymph nodes (Fig. 1). When set off by various alarm systems of the innate immune response to infection, such as by interferons, tumor necrosis factor α (TNFα) or bacterial lipopolysaccharide, these cells quickly bring antigens that they have captured into the lymphoid parenchyma of the secondary lymphoid tissues [2–4].

Dendritic cells that have been aroused by infection or inflammation take up residence in the T-cell (T) zone, one of the two key compartments of the lymphoid parenchyma (Fig. 1) [2,3]. Antigens that have been captured and then presented by dendritic cells in the T zone can be efficiently surveyed by many T cells in a short time, because of an orchestrated, continuous flow of recirculating T cells passing from the blood through this zone. In the spleen, T cells exit the blood in the marginal zone and migrate into the T zone for less than a day before exiting back into the blood, probably by returning to the marginal sinus [3]. In lymph nodes and Peyer's patches, the T cells leave the blood by extravasating across specialized high endothelial venules, again migrating amongst the dendritic cells of the T zone before exiting into the lymph fluid of the medullary sinus, where they are swept back into the circulation.

In each organ, the bulk of the T-cell population will not have been triggered by an antigen and remains on the move, returning to the circulation to continue immunological surveillance without stopping and without entering the follicles, the other chief compartment of secondary lymphoid organs. Intermixed with this flux of T cells, however, is an equally large throughput of B cells that are also on continuous surveillance. Like T cells, recirculating B cells also leave the blood in the marginal zones of spleen and *via* high endothelial venules in lymph nodes and Peyer's patches [3]. Initially, B cells follow an overlapping track with T cells through the T zone, but within several hours of reaching this site they divert into follicles, where they become concentrated for about a day before exiting back into circulation. A different class of dendritic cells, the follicular dendritic cells, forms a meshwork among the

Figure 1



Simplified depiction of lymphoid tissue architecture and cell migration routes in spleen, lymph nodes and Peyer's patches. For the spleen, the diagram represents a single white pulp cord with the surrounding red pulp stripped away. Lymphocytes are released in the spleen from terminal arterioles that branch from central arterioles (ca) and frequently end in the marginal sinus that surrounds the lymphoid compartments. Lymphocytes enter lymph nodes and Peyer's patches across specialized high endothelial venules (hev), usually located in the T-cell zone. Red arrows indicate fluid and antigen movement (blood, lymph or intestinal fluid transcytosed by M cells); green arrows show T-cell migration; and blue arrows show B-cell migration. Blood leaves the spleen *via* venous sinuses in the red pulp that are not shown. Shaded B and T cell zones represent areas tightly packed with predominantly B or T cells, respectively. See text for further details of lymphocyte and antigen traffic.

B-cell-rich follicles. Follicular dendritic cells specialize in presenting antigens to B cells [5], after capturing antigen either directly or more likely by transfer from ill-defined 'antigen transport cells' that may correspond to marginal zone/sinus-lining macrophages and B cells [4].

The bifurcating traffic of T and B cells through secondary lymphoid tissues has one chief functional result: to guide efficiently a large repertoire of different T and B cells past antigen-capturing cells that are specialized for presenting antigen to the respective cell types. During a new infection or immunization, antigen is presented initially by dendritic cells in the T zone. Rare T cells that carry T cell receptors for the presented antigen are triggered by signals from their receptors to halt their recirculation and begin proliferating in the T zone [3,6,7]. Rare B cells that carry surface immunoglobulin antigen receptors for the foreign antigen can encounter the antigen in a variety of sites, either in the follicles or T zone, or in the sinuses or on sinus-lining macrophages. In either case, engagement of their antigen receptors triggers these B cells selectively to halt their surveillance traffic pattern and actively seek out the T zone, where they stop in amongst T cells and dendritic cells [8–11]. Rare B and T cells that have specifically bound to a foreign antigen therefore alter their traffic patterns and congregate in the T zone, where the T cells, after activation by dendritic cells, can help the specific B cells to expand clonally and begin to form secreted antibody against the immunogen [3,12].

As an immune response develops following infection or immunization, some of the T and B cells that had been congregating and proliferating in the T zone change their tropism once more. The 'memory' T cells now begin to enter the normally exclusive club of B cells, namely the follicles [7,13]. The primed B cells soon follow and begin proliferating rapidly in the follicle, forming a subdomain called a germinal center [8,12]. Within this structure, the antigen receptors on the B cells undergo intense hypermutation and selection for higher affinity and specificity against the foreign antigen, presumably by testing the fit of their receptors against antigens presented by the follicular dendritic cell meshwork. This second, follicular phase of the immune response appears to be essential for the normal development of a persistent, high-titer antibody response and an accelerated memory response to re-infection.

Until the recent work of Förster *et al.* [1], little was known about the specific cues that guide these orchestrated movements of B and T cells between the T zone and follicles. By contrast, the molecular 'addressins' that regulate the initial egress of T and B cells out of the bloodstream, across high endothelial venules and into the lymphoid parenchyma have begun to be elucidated [14,15]. That work has revealed a complex interplay between carbohydrate-recognizing selectins that promote rolling along the endothelium, chemokines that signal through G-protein coupled receptors, and integrins that mediate strong adhesion in response to chemokine-receptor signals. The further steps in the migratory loop of T and B cells into the T zone and follicles, or the redistribution of antigen-primed cells between T zones and follicles, essentially remained a black box. A G-protein-mediated signal was implicated in these steps, because pertussis toxin abolishes

migration of recirculating T and B cells from the marginal zone into the T zone and follicles of the spleen [16,17].

The function of Burkitt's lymphoma receptor 1

Lipp's group initially cloned a novel cDNA, dubbed *BLR1*, in a subtractive screen for genes that were differentially expressed in a type of B-cell tumor called Burkitt's lymphoma [18]. They noted that the BLR1 protein bore sequence homology to receptors for chemotactic molecules of the innate immune system, such as the receptors for interleukin 8, for the C5a fragment of complement, or for *N*-formylated peptides from bacteria. These receptors are all of the type characterized by having seven transmembrane-spanning domains, and signal by activating heterotrimeric G-proteins that are predominantly sensitive to pertussis toxin. Many of the newer members of this receptor family are known to bind small chemotactic proteins called chemokines, although the ligand for BLR1 remains unknown. Chemokines are very basic proteins that bind to anionic extracellular matrix components, such as heparin sulphate, where they may form an immobilized chemotactic gradient [19,20].

The first strong hint that BLR1 might be involved in follicular homing of lymphocytes came from its unique pattern of expression, as revealed by staining human cells with a specific monoclonal antibody [21]. Strikingly, BLR1 protein was absent from immature B cells in the bone marrow, but was expressed abundantly on mature recirculating B cells at precisely the stage in their development when they become follicle-seeking. Moreover, BLR1 protein was not present on most recirculating T cells, but was expressed on a subset of memory T cells, some of which were located in follicles [21].

The function of BLR1 has now been tested in mice by inactivating the gene *via* homologous recombination in embryonic stem cells [1]. Recirculating B cells still develop and mature in mice that are homozygous for a null mutation of the *BLR1* gene, but when these B cells are tagged and introduced into the bloodstream of a normal mouse, they fail to home properly to the follicles of spleen and Peyer's patches. Instead, the BLR1-deficient B cells remain at the periphery of the T zones of these organs. While follow-up kinetic studies will be important, this result alone establishes that BLR1 is an important homing receptor for follicle-seeking behavior in B cells, presumably by recognizing an unknown chemotactic gradient into the follicles. Which cells produce the BLR-1 ligand, and whether it is attractive or repulsive, are questions that follow immediately from this result.

Mice that lack BLR1 also exhibit a more generalized abnormality in the development of their secondary lymphoid organs [1], indicating a possible second role for BLR1. Thus, $BLR1^{-/-}$ mice lack inguinal lymph nodes, have few Peyer's patches, and recirculating (IgD^+) B cells in the spleen are not organized into discrete follicles but form a thin band inside the marginal sinus. Following immunization, germinal centers fail to develop in the usual sites in the spleen, although collections of activated B cells did form within the T zone. The staining patterns in the spleen suggest that the immune-complex-bearing follicular dendritic cells are missing, these cells being specialized for presenting antigens to B cells in follicles and germinal centers. The failure to develop normal follicles, follicular dendritic cells and germinal centers in spleen could reflect a function of BLR1 in B cells not only to guide them to the correct compartment but also to stimulate them to make a factor that promotes development of follicular dendritic cells. There is good evidence that the latter develop from fibroblastic reticular cells in the lymphoid stroma, and that their differentiation into antigen-capturing follicular dendritic cells depends upon signals from B cells [22].

While not commented upon by Förster *et al.* [1], the developmental defect in BLR1-deficient mice almost perfectly mirrors the defects very recently described in mice that have been made genetically deficient in the membrane-bound and secreted cytokine, TNFα [23]. These mice also lack Peyer's patches, show a thin rim of peripheral IgD^+ B cells in the spleen without follicles or follicular dendritic cells, lack germinal centers and form misplaced accumulations of activated B cells in the T zone. A similar phenotype also occurs in mice lacking one of the receptors for TNFα, TNFR1 [24,25]. Taken together, it might be speculated that BLR1 not only attracts B cells to follicular niches but also stimulates them to make TNFα, in turn promoting differentiation of the follicular dendritic cell meshwork needed for high-titer antibody responses.

Surprisingly, BLR1-deficient B cells still home normally to follicles in lymph nodes, and germinal centers form normally in these organs [1], indicating that BLR1 is not the only follicular addressin. One inference from this finding is that a different chemotactic gradient guides B cells to follicles in lymph nodes compared with spleen and Peyer's patches. Additional B-cell guidance mechanisms are also implicated in the latter organs, because the BLR1-deficient B cells that fail to gain access to follicles are not distributed randomly but congregate at the periphery of the T zone, near the sites where sinus-lining macrophages and resting dendritic cells are found. Possible candidates for mediating these effects exist. For example, recirculating B cells also respond chemotactically to another chemokine, stromal-derived factor/pre-B cell stimulating factor 1, using a BLR1-related chemokine receptor called CXCR4 [26]. The latter receptor is also carried by T cells, where it is implicated in their migration as well as functioning as a co-receptor for human immunodeficiency virus 1 (HIV-1). The precise migratory path of lymphocytes through secondary

lymphoid tissue may thus reflect a complex interplay of response and desensitization to different chemokines.

Implications for disease

A range of important issues begin to become experimentally and perhaps therapeutically tractable following the finding that chemokine-like receptors guide follicular development and homing. In immunity and immunodeficiency, it has become clear that follicles are a major repository for infectious HIV-1 virions, and that preferential migration of memory CD4 T cells to these sites may be a critical step in the evolution of AIDS. In the area of autoimmunity, peripheral tolerance in T cells [13] and in self-reactive B cells [9,10] has, in each case, been linked to the selective exclusion of these cells from follicles. Because that process in self-reactive B cells depends on binding a critical threshold of self antigen and competition between B cells [9,10], a decrease in expression or function of receptors for follicular chemokines had been hypothesized to occur on self-reactive cells [27]. Competition between B cells to regulate the size and composition of the recirculating repertoire may be achieved by the highest receptor-bearing cells gaining preferential access to follicles and then steepening the chemotactic gradient by mass action. This selective mechanism would be analogous to the apparent effect of high Patched receptor expression on the gradient of its putative ligand, Hedgehog, in *Drosophila* development [28].

Ectopic formation of follicles and germinal centers occurs in tissues that are afflicted by chronic autoimmune reactions, such as in the pathologic synovium of rheumatoid arthritis patients or the thyroid glands of individuals with Grave's disease. Similarly, neoplastic lymphomas of B cell origin frequently retain their tropism for follicular structures, driving the formation of pathologically large and numerous follicles in secondary lymphoid tissue and in extranodal sites. A detailed understanding of the molecules guiding follicular migration may illuminate the pathogenesis of these diseases and reveal targets for the development of new therapeutics.

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